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EFFECT OF MANURE-SULPHUR COMPOSTS UPON THE AVAILABILITY OF THE POTASSIUM OF GREENSAND

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INTRODUCTION

The greensands and the greensand marl deposits of the eastern United States have long been regarded as a possible source of potassium for agricultural purposes. The literature of the last half of the nineteenth century contains many reports of the success that has followed the application of greensand marls to soils in Maryland, New Jersey, and other eastern States. Since many of these marls contain a high percentage of calcium carbonate, it is probable that the good results that followed their use was due in many cases to their lime content rather than to the potassium which they contained.

During the continuance of the war with Germany, the scarcity and the consequent high price of readily soluble potassium salts has served to direct attention in this country to the possibility of utilizing for agricultural purposes the potassium of these greensand deposits and has indicated the desirability of devising some efficient method of treatment that would render the potassium more available. At the suggestion of the fertilizer committee of the National Research Council, the Department of Soil Investigations of this Station has studied the effect of composting greensand with sulphur, manure, and other materials with a view to making available the potassium contained in the greensand. It is the purpose of this paper to report the results of this investigation.

HISTORICAL

As early as 1830 Thomas Gordon called attention to the great benefits that farmers in New Jersey were deriving from the use of marl. In a geological report published in 1868, Cook (6)[1] gives the analyses of a number of samples of marl from New Jersey and states that the use of this material has raised the land from a low state of exhaustion to a high stage of agricultural development. He states that some of these marls are so acid that heavy applications of as much as 50 tons to the acre

[1] Reference is made by number (italic) to "Literature cited," p. 255-256.

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Key No. Md.-2

have been known to destroy all vegetation and advises that the use of such marls should be confined to well-limed land or that they should be composted with lime before being applied. In 1906 Patterson (*12*) published the results of the examination of 95 samples of Maryland marl. In summing up the results of his experimental work covering a period of 11 years this writer concludes that the shell marls of Maryland have very little commercial value because of the great bulk of worthless material contained in them but that they should have considerable local agricultural value, both as a source of lime and also for the potassium which they contain. He concludes that while much of the potassium in marls will become slowly available to plants through weathering, the change necessary to liberate the potassium could readily be brought about by burning the calcarious marls and slaking the product.

In a popular discussion of the agricultural value of greensand marl Blair (*3*) concludes that since potassium is of especial value to grass and to potatoes, the striking benefits derived from the use of marl on these crops would lead to the belief that such crops can use the potassium of the marl to a considerable extent.

From pot experiments carried out with crushed quartz and Shive's cultural solution as a basis, True and Geise (*13, p. 492*) conclude that—

> greensands and greensand marls from Virginia and New Jersey are able to supply sufficient potassium to satisfy the demands of Turkey Red wheat and red clover during the first two months of their growth.

They secured a greater dry weight of tops from cultures containing greensand marl than from those in which the potassium demand was supplied by potassium chlorid, potassium sulphate, or potassium phosphate. These results are in harmony with those reported by Lipman and Blair (*8*) who found that soybean plants fertilized with greensand produced as great a yield of hay as those receiving an application of soluble potassium salts, although the former failed to produce seed. These last-mentioned authors hold that their results seem to furnish proof of the ease with which the soybean gets its potash from slowly available sources up to the time the beans are forming and maturing. In the same report these writers describe another experiment in which Canada field peas and soybeans growing in sand cultures were given a general fertilizer treatment to which was added marl containing 6.5 per cent of potash. Two pots in this series received 20 gm. of marl, while two additional pots received in addition to the 20 gm. of marl, 3 gm. of sulphur each, with the thought that the oxidation of the sulphur might result in making more of the potash of the marl available. The Canada field peas were grown as the first crop, followed by the soybeans as a second crop. Both pots receiving the sulphur treatment gave very much decreased yields of field peas, and in one of the duplicates the soybeans that followed the peas failed completely. The other duplicate, however, gave a yield of soybeans slightly in excess of that produced by any of the other treat-

ments, including the pots receiving 2 gm. of potassium chlorid. In their conclusions they suggest—

the possibility of utilizing the potash of greensand marl and the potash of natural soil materials by growing soybeans and possibly certain other crops, which could be returned to the soil and thus furnish available potash for those crops which can not readily utilize potash from these natural sources.

Lipman, McLean, and Lint (*10*) composted 100-gm. portions of sea sand, sassafras loam, and greenhouse soil with manure, sulphur, and floats. At the end of 30 weeks analyses for water-soluble phosphoric acid showed increases in all the mixtures to which both sulphur and floats had been added. In one case 85 per cent of the total phosphorus in the floats had been made available, the increase in available phosphorus paralleling the oxidation of the sulphur as measured in terms of sulphates. In experiments conducted under field conditions, two of these authors (*9*) have shown that the sulphur-floats-soil compost may be utilized in making available the phosphorus of floats or raw ground phosphate rock. They suggest that this compost could be employed to advantage as a substitute for acid phosphate. Further studies at the New Jersey Experiment Station by McLean (*11*) led to the conclusion that the most economical combination for the production of available phosphoric acid is a compost composed of 100 parts soil, 120 parts sulphur, and 400 parts floats.

Brown and Warner (*5*) found that by composting floats with manure and sulphur it was possible to obtain a remarkable increase in the amount of available phosphoric acid. The increase was greater where the sulphur and floats were intimately mixed with the manure than where the material was arranged in alternate layers.

Experimenting with two Iowa soils, Brown and Gwinn (*4*) found that while applications of manure alone increased the availability of raw rock phosphate, the increase was much greater when sulphur was used in connection with the manure. They bring out the fact that there is a definite relationship existing between the sulphofying power of the soil and the production of available phosphorus.

Ames and Richmond (*2*) found that in an acid soil oxidation of sulphur proceeded vigorously, approximately 50 per cent of the sulphur being changed to the form of sulphate. In a basic soil the acidity resulting from sulphofication was partly neutralized, so that the solvent action on the rock phosphate was much less than occurred in the acid medium.

Since the inauguration of our work, Ames and Boltz (*1*) have published additional data concerning the effect of sulphur on soils and crops. These investigators found that both the nitrification of dried blood and the oxidation of sulphur in soil mixtures resulted in the liberation of potassium. They conclude that the liberation of the potassium was brought about by the salts formed rather than by the direct action of acidity on the insoluble potassium compounds.

PURPOSE AND PLAN OF THE INVESTIGATION

With the foregoing results in mind the present investigation was undertaken for the purpose of determining the effect of different composts upon the availability of the potassium of greensand. The investigation consisted of composting greensand with sulphur, soil, and manure in varying proportions, taking samples from time to time, extracting these samples with distilled water and analyzing the water extracts for the acidity, sulphate, and potassium contained.

Two series of composts were conducted, one series containing a greensand from Sewell, N. J., having a relatively high percentage of potassium, and the other a greensand from Crownsville, Md., having a rather low percentage of potassium. Each compost contained as a basis 1,500 gm. of greensand. The materials added were the same for each series and were as follows:

COMPOST NO.	MATERIALS ADDED TO GREENSAND.
1 and 8	Nothing.
2 and 9	500 gm. sulphur.
3 and 10	500 gm. sulphur; 500 gm. manure.
4 and 11	500 gm. sulphur; 250 gm. manure; 250 gm. soil.
5 and 12	500 gm. sulphur; 500 gm. soil.
6 and 13	500 gm. sulphur; 500 gm. soil; 0.02 per cent aluminum sulphate ($Al_2(SO_4)_3$) 0.18 H_2O; 0.02 per cent ferrous sulphate ($FeSO_4$) 0.7 H_2O.
7 and 14	500 gm. sulphur; 250 gm. soil; 250 gm. manure; 10 gm. calcium carbonate ($CaCO_3$).

Commercial flowers of sulphur, partially rotted yard manure air-dried and ground fine, Collington sandy loam, and precipitated calcium carbonate were used. The aluminum and ferrous sulphates were added to composts 5 and 12 in order to determine whether these salts would exert a stimulating effect upon the rate and amount of sulphofication. McLean (*11*) found that, under certain conditions, these salts in combination exerted a marked stimulating action on sulphur oxidation processes when present in small amounts. He advocated the use of 0.4 pound per ton, or 0.02 per cent, of each for sulphur-floats composts. It was thought desirable to ascertain whether this effect would be obtained with sulphur-greensand composts.

METHODS OF PROCEDURE

The air-dry materials for each compost were weighed and thoroughly mixed. Similar smaller amounts of the same materials were mixed in the same proportions, from which the moisture-holding capacity of each compost was determined according to the Hilgard method (7, *p. 209*).

After being mixed, each compost was placed in a glazed pot, and water was added to one-half the determined water-holding capacity. The samples for the first analyses, showing the amounts of water-soluble

acidity, sulphate, and potassium at the start, were then taken, after which each compost was inoculated with the sulphofying organisms, and the aluminum and ferrous sulphates were added in solution to composts 6 and 13.[1]

The period of composting was 23 weeks. Once each week the amount of water lost by evaporation was added, and the composts were removed from the pots and mixed, in order to provide thorough aeration.

The composts were kept in the greenhouse throughout the entire period and were covered at all times with a double thickness of white muslin to protect them from direct sunlight. The temperature of the greenhouse ranged from 50° to 100° F.

For the water extraction a 75-gm. sample was weighed from each compost, air dried, and 50 gm. of the air-dry material were shaken every half hour for 8 hours with 500 cc. of distilled water in a 1-liter Pyrex flask. After standing over night, the contents of the flasks were again shaken and filtered rapidly through folded No. 3 Whatman filter papers. The first 100 cc. of filtrate were poured back. The filtrates obtained were absolutely clear and free from sediment.

The acidity was determined by boiling aliquots of the water extract to expel carbon dioxid, cooling, and titrating with *N/10* sodium hydroxid, in terms of which the results are stated. Phenolpthalein was the indicator used. Titration was continued until all soluble iron, aluminum, and silica were precipitated and the clear solution retained the pink color for one minute.

Sulphur was determined by acidifying aliquots of the water extract with 2 cc. of concentrated hydrochloric acid and precipitating at the boiling point with barium chlorid. The results are expressed as sulphur trioxid (SO_3).

The potassium determinations were made gravimetrically by the platinic chlorid method from aliquots of the water extract, first eliminating the soluble organic matter, silicates, iron, aluminum, and phosphorus by evaporation with sulphuric acid, ignition, and subsequent precipitation. The determination for composts 1 and 8 throughout and the first three determinations for the other composts not containing manure were made colorimetrically because of the small amounts of potassium present.

Moisture determinations were made by heating separate 5-gm. portions of the air-dry compost for 15 hours at 105° C. All results reported in this paper are calculated to the moisture-free basis. No duplicate determinations were made, the idea being that one series of compost treatments would act as a control for the other in regard to the general trend of the reaction and that any serious error in analysis would

[1] Cultures containing sulphofying organisms were supplied by Dr. J. G. Lipman and Prof. A. W. Blair, of the New Jersey Experiment Station.

readily be shown and offset by the frequency with which the analyses were made.

The greensands, soil, and manure used were analyzed at the beginning of the investigation. The results are given in Table I. The potassium determinations were made by the official fusion method.

TABLE I.—*Composition of materials used (dry basis)*

Materials.	Moisture at 105° C.	Insoluble residue.	Ferric oxid (Fe_2O_3), aluminum oxid (Al_2O_3), phosphorus pentoxid (P_2O_5).	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium (K).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
New Jersey greensand	5.46	53.12	31.66	0.16	1.05	5.88
Maryland greensand	1.57	87.83	8.38	.13	.25	1.42
Collington sandy loam	1.20	89.54	7.54	.18	.22	.83
Manure [a]	6.30					.49

[a] Loss on ignition, 69.67 per cent.

Determinations made by the Veitch method showed that the New Jersey greensand required 4,200 pounds of calcium carbonate per 2,000,000 pounds, the Maryland greensand 3,400 pounds, and the Collington sandy loam 1,400 pounds.

The texture of the greensands and soil is shown in Table II, which gives the mechanical analyses of the materials used in the composts.

TABLE II.—*Mechanical analyses of greensands and soil*

Constants.	New Jersey greensand.	Maryland greensand.	Collington sandy loam.
	Per cent.	*Per cent.*	*Per cent.*
Fine gravel	4.94	1.49	0.53
Coarse sand	30.26	1.77	10.14
Fine sand	45.22	9.15	26.27
Very fine sand	15.14	83.65	43.93
Silt and clay	4.11	3.86	18.72

PRESENTATION AND DISCUSSION OF RESULTS

ACIDITY

In Table III is shown the acidity of the water extract from each compost as determined at the end of each 1-week period for the first 9 weeks and thereafter at the end of each 3 weeks for a total period of 23 weeks. The results are expressed in terms of *N/10* sodium hydroxid required to neutralize the acidity in the water extract from 10-gm. of compost on the dry basis.

TABLE III.—*Accumulation of water-soluble acidity*

Basis.	Compost No.	Materials added to 1,500 gm. greensand.	Cubic centimeters *N*/10 sodium hydroxid required to neutralize acidity of water extract from 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks.	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand	1	None	0.05	0.075	0.075	0.075	0.075	0.075	0.10	0.05	0.05	0.075	0.05	0.05	0.05	0.05	0.075
	2	Sulphur 500 gm	.05	.075	.10	.50	1.05	1.20	2.50	1.35	1.90	2.30	2.85	3.50	3.50	3.60	4.50
	3	Sulphur 500 gm.; manure 500 gm	.05	.60	.70	3.50	8.05	17.20	36.75	59.15	151.35	156.50	146.25	126.20	151.00	157.85	159.20
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	.05	.25	.45	3.55	7.10	15.30	29.54	37.20	42.90	45.70	79.70	95.65	97.05	94.10	96.60
	5	Sulphur 500 gm.; soil 500 gm	.05	.075	.10	.65	1.35	1.90	2.55	3.10	3.70	4.80	7.30	12.15	18.95	25.10	35.45
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	.05	.075	.10	1.00	2.45	3.15	4.45	5.10	5.85	7.25	10.20	17.10	24.80	28.60	33.35
	7	Sulphur 500 gm.; soil 250 gm.; maure 250 gm.; $CaCO_3$ 10 gm.	Alk.	.50	.60	1.50	38.25	65.00	62.10	59.80	61.25	64.40	97.65	105.40	104.95	102.80	101.35
Maryland greensand	8	None	.05	.05	.05	.05	.075	.075	.10	.075	.05	.075	.05	.05	.05	.05	.05
	9	Sulphur 500 gm	.05	.05	.075	.35	.70	.95	1.30	1.30	1.90	2.05	2.80	3.75	4.35	4.05	5.15
	10	Sulphur 500 gm.; manure 500 gm	.05	.70	.65	1.25	6.65	17.65	37.45	50.90	141.20	156.55	147.80	126.65	135.65	132.65	134.15
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	.05	.75	.70	2.90	7.30	18.05	30.40	37.80	42.95	73.20	112.85	111.10	114.85	110.55	116.85
	12	Sulphur 500 gm.; soil 500 gm	.05	.05	.15	.90	1.75	2.40	3.05	4.00	4.70	5.85	9.15	14.80	24.05	32.40	41.00
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	.05	.075	.10	.45	1.55	2.10	2.80	3.45	4.25	5.10	8.55	15.20	24.30	28.25	36.20
	14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	Alk.	.075	.75	1.30	6.55	10.90	26.70	57.50	103.90	104.00	103.35	107.40	108.75	108.85	112.15

Attention is called to the fact that, although both greensands showed a high lime requirement when tested by the Veitch method, neither of them gave evidence of more than a trace of acidity in the water extract. The addition of sulphur to the greensand in the proportion of 3 parts greensand to 1 part sulphur caused a gradual accumulation of water-soluble acidity, because of the slow oxidation of the sulphur. Composts 3 and 10, in which both sulphur and manure were mixed with the greensand, show a slight and gradual accumulation of water-soluble acidity up to the end of the fifth week, after which there is a very rapid rise for three weeks. For the remainder of the period the acidity fluctuates at a high and practically constant level. When one-half of the manure is replaced by an equal quantity of soil, as in composts 4 and 11, the acidity is greatly reduced, the maximum for the Maryland greensand being reached at the end of the 12-week period and for the New Jersey greensand after 15 weeks. When the manure was entirely replaced by soil, the acidity increased gradually throughout the entire period, as shown by composts 5 and 12; but the amount developed was only about one-third as much as when equal weights of soil and manure were used. This indicates rather strongly that in composts made up with a greensand deficient in calcium carbonate the rate of development and the amount of acidity depend very largely on the amount of organic matter present. A further comparison of composts 5 and 12 with 2 and 9 seems to substantiate this conclusion, in that the soil used contained a small amount of organic matter.

The acidity titrated did not, of course, at any time consist entirely of free sulphuric acid. As sulphofication progressed and the amounts of free sulphuric acid and sulphates increased, an increasing amount of acid silicates was obtained in the water extract and was precipitated upon titration with the alkali. Careful inspection of several titrations, made after the maximum acidity had been attained, seemed to indicate that from 45 to 55 per cent of the acidity titrated was due to free sulphuric acid, the remainder of the acidity being due to acid silicates and other acid salts.

Under the conditions of our experiment the addition of ferrous sulphate and aluminum sulphate when used at the rate recommended by McLean (*11*) for sulphur-floats composts has had no appreciable effect, as may be seen by a comparison of composts 6 and 13 with 5 and 12. The addition of 10 gm. of calcium carbonate to the sulphur-manure-soil compost had a marked stimulating effect, beginning about the third week in the New Jersey greensand compost and two weeks later in the Maryland greensand compost. In the former the stimulating action persisted up to the end of the experiment, while in the latter the effect of the calcium carbonate had entirely disappeared at the end of 12 weeks. A cause for this difference is found when the lime requirement of the New Jersey greensand is

compared with that of the Maryland greensand. As was previously mentioned, the lime requirement of the New Jersey material is 4,200 pounds of calcium carbonate, while for the Maryland greensand the requirement is only 3,400 pounds. The results recorded in Table III would appear to justify the conclusion that an initial acidity corresponding to a lime requirement of 3,400 pounds of calcium carbonate exerts a slightly depressing effect upon sulphofication, and that an acidity corresponding to a lime requirement of 4,200 pounds of calcium carbonate is less favorable. Ames and Boltz (*1*) found that calcium carbonate when added in excess of the lime requirements exercised a depressing effect upon the oxidation of sulphur in their soil-sulphur compost. When they reduced the application to half, the oxidation of sulphur increased but was less than when no carbonates were added.

SOLUBLE SULPHATES

A comparison of the results recorded in Table IV with those given in Table III shows that the accumulation of water-soluble sulphates parallels very closely the development of acidity.

It will be observed that the sulphur trioxid determinations fluctuate somewhat after having attained a maximum at the end of about 12 weeks. These fluctuations are probably due to variations in the moisture content and the temperature of the composts, since such variations are known to have an effect upon colloidal silicates, which in turn might exercise, through adsorption, an appreciable effect upon the soluble sulphur trioxid obtained in the water extraction. A calculation shows that at the end of our 23-week period, approximately 15 per cent of the total sulphur used in composts 3 and 10 had been oxidized, while for the composts in which one-half of the manure had been replaced by soil about 11 per cent of the total sulphur had been oxidized. These figures show that the amount of sulphur used was in excess of the amount necessary to secure the most economical results.

SOLUBLE POTASSIUM

The amount of water-soluble potassium in each compost at stated intervals is given in Table V.

A comparison of these figures with those given in Tables III and IV brings out the fact that with the increase in acidity and the accumulation of sulphur trioxid there is a corresponding increase in the amount of potassium in the water extract. The potassium, however, continues to increase for some weeks after the acidity and sulphur trioxid have reached a maximum. It seems necessary for a certain degree of acidity to be developed before any appreciable amount of potassium is made water soluble, the larger amounts of acidity and soluble sulphate breaking down the greensand more rapidly.

TABLE IV.—*Accumulation of water-soluble sulphate*

Basis.	Compost No.	Materials added to 1,500 gm. greensand.	Milligrams water-soluble sulphur trioxid (SO_3) in 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks.	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand.	1	None	0.68	0.93	0.98	1.36	1.79	1.58	1.69	1.87	1.94	1.51	1.73	1.91	1.61	1.73	1.76
	2	Sulphur 500 gm.	1.07	1.57	1.89	8.45	11.94	13.59	14.62	15.82	17.63	18.91	22.99	25.33	26.93	27.47	31.92
	3	Sulphur 500 gm.; manure 500 gm.	4.68	22.70	34.67	68.59	93.85	133.60	206.60	298.98	660.99	714.69	698.99	638.28	740.72	811.80	812.09
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.57	7.72	17.75	47.03	64.44	100.41	156.44	187.27	213.53	224.87	382.81	468.39	473.47	475.67	485.74
	5	Sulphur 500 gm.; soil 500 gm.	1.07	1.49	2.98	10.54	14.38	18.01	21.32	24.88	28.04	32.10	42.28	61.76	89.13	116.35	161.87
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	1.07	2.41	2.56	13.06	19.00	24.46	30.32	34.90	40.02	42.70	56.24	81.63	114.93	130.62	152.77
	7	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	3.50	23.25	47.61	59.66	213.11	320.30	313.29	309.21	317.44	330.69	492.98	543.58	549.24	546.50	535.55
Maryland greensand.	8	None	.42	.91	.84	1.69	1.47	1.65	1.79	1.82	2.43	2.00	2.56	2.46	2.14	2.31	2.14
	9	Sulphur 500 gm.	.98	1.57	2.28	7.88	9.91	11.38	13.40	15.01	16.55	17.43	21.41	25.64	27.57	26.31	30.66
	10	Sulphur 500 gm.; manure 500 gm.	4.84	26.16	34.30	46.64	84.90	136.33	211.75	266.28	609.83	723.14	674.56	632.02	673.54	668.98	672.47
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.53	14.39	21.90	42.52	66.87	107.93	158.88	186.82	212.05	329.96	522.00	544.68	558.13	542.11	568.02
	12	Sulphur 500 gm.; soil 500 gm.	.87	1.81	4.91	11.35	16.54	19.63	23.05	27.71	31.89	35.34	47.69	71.78	107.82	143.76	178.12
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	.80	2.09	3.10	8.47	14.62	17.52	22.45	25.14	29.24	32.30	44.18	72.85	109.26	125.09	158.08
	14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	4.21	26.92	51.58	55.80	85.87	107.19	153.91	291.09	478.97	506.35	516.51	544.41	551.75	558.37	577.65

TABLE V.—*Accumulation of water-soluble potassium*

Basis.	Compost No.	Material added to 1,500 gm. greensand.	Milligrams water-soluble potassium in 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks.[a]	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand	1	None	0.19	0.30	0.22	0.24	0.18	0.41	0.45	0.46	0.41	0.31	....	0.42	0.41	0.49	0.45
	2	Sulphur 500 gm.	.24	.29	.45	.62	.98	.87	1.05	1.12	.98	1.20	....	1.26	1.30	1.17	1.43
	3	Sulphur 500 gm.; manure 500 gm.	2.99	5.75	7.43	9.87	8.05	11.57	10.11	11.68	14.77	17.64	....	40.04	48.46	62.56	64.11
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	1.47	2.70	3.56	5.55	4.68	5.85	5.99	6.10	8.98	8.18	....	25.91	31.25	30.10	32.77
	5	Sulphur 500 gm.; soil 500 gm.	.32	.32	.48	.72	1.10	.87	.91	.96	1.07	1.02	....	1.30	2.08	3.18	3.36
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	.34	.34	.44	.75	1.05	.87	.97	1.17	1.16	1.27	....	1.34	2.25	3.10	3.36
	7	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	1.78	3.08	5.41	5.85	5.13	10.03	9.42	9.53	11.08	10.36	....	29.89	31.94	39.39	33.74
Maryland greensand	8	None	.16	.21	.29	.27	.41	.48	.46	.52	.50	.36	....	.41	.46	.48	.48
	9	Sulphur 500 gm.	.26	.24	.56	.52	.98	.72	.96	.99	.93	.96	....	.84	1.28	1.02	1.08
	10	Sulphur 500 gm.; manure 500 gm.	3.09	7.18	8.57	8.35	7.96	10.70	10.95	11.39	15.51	16.53	....	33.62	39.65	39.23	37.11
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.54	3.48	4.15	5.33	4.37	5.80	6.04	6.21	9.25	8.36	....	23.74	28.02	26.75	28.16
	12	Sulphur 500 gm.; soil, 500 gm.	.30	.30	.64	.65	1.03	.85	1.02	1.03	.88	1.10	....	.96	1.58	1.17	1.21
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O.	.28	.36	.56	.76	.84	.76	.84	.91	1.06	.99	....	.93	1.26	.95	.82
	14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	2.30	4.22	6.27	5.86	4.77	6.90	5.71	6.35	11.97	10.22	....	23.46	25.26	25.33	26.53

[a] No analyses made.

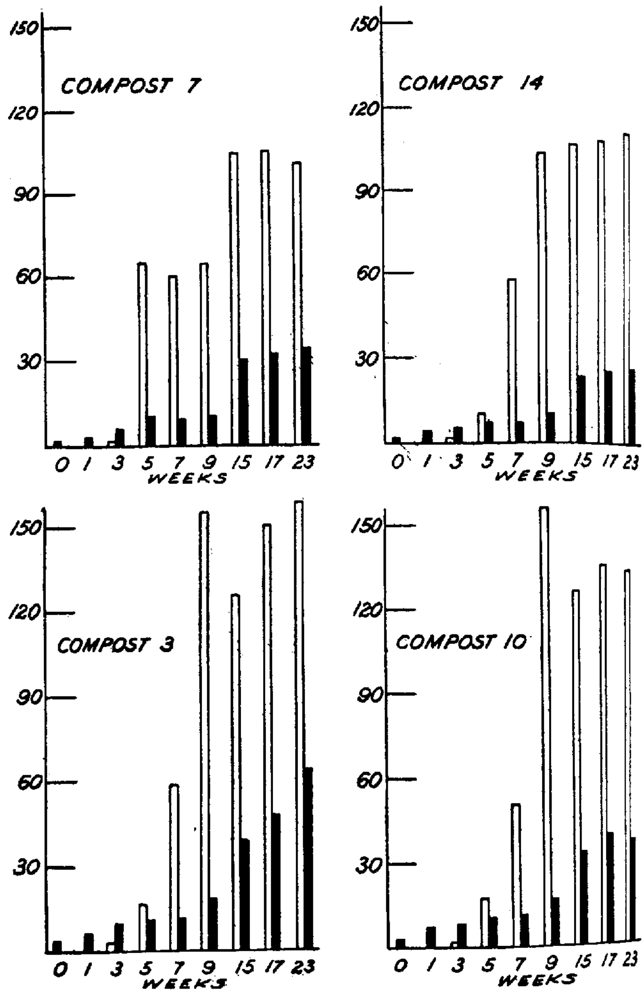

FIG. 1—Diagrams showing relation of the water-soluble acidity to the water-soluble potassium at different time periods for different greensand composts. The open columns indicate the number of cubic centimeters of *N*/10 sodium hydroxid required to neutralize 10 gm. of compost on moisture-free basis, and the solid columns indicate the number of milligrams of water-soluble potassium obtained from 10 gm. of compost on moisture-free basis.

The diagrams of figure 1 give a graphic representation of the relation of the water-soluble acidity to the water-soluble potassium at different time periods for the greensand-sulphur-manure composts and for the greensand-sulphur-soil-manure composts to which were added 10 gm. of calcium carbonate.

A comparison of compost 3 with compost 7 and compost 10 with compost 14 brings out the fact that the replacement of one-half of the manure by soil has reduced the acidity and at the same time decreased the amount of potassium in the water extract. No. 7 and 14 show also the stimulation in acidity during the early weeks due to the addition of calcium carbonate.

The degree of acidity and the amount of sulphates and of potassium in the water extracts at the beginning of the period and at the end of 23 weeks for all the composts are shown in Table VI, which is a summary of Tables III, IV, and V.

TABLE VI.—*Water-soluble acidity, sulphate, and potassium in water extract from 10 gm. of moisture-free compost at beginning and after 23 weeks of composting*

Basis.	Compost No.	Acidity (cc. N/10 sodium hydroxid required).		Sulfate (sulphur trioxid).		Potassium.	
		After 0 weeks.	After 23 weeks.	After 0 weeks.	After 23 weeks.	After 0 weeks.	After 23 weeks.
				Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
New Jersey greensand....	1	0.05	0.075	0.68	1.76	0.19	0.45
	2	.05	4.50	1.07	31.92	.24	1.43
	3	.05	159.20	4.68	812.09	2.99	64.11
	4	.05	96.60	2.57	485.74	1.47	32.77
	5	.05	35.45	1.07	161.87	.32	3.36
	6	.05	33.35	1.07	152.77	.34	3.36
	7	Alkaline	101.35	3.50	535.55	1.78	33.74
Maryland greensand......	8	.05	.05	.42	2.14	.16	.48
	9	.05	5.15	.98	30.66	.26	1.08
	10	.05	134.15	4.84	672.47	3.09	37.11
	11	.05	116.85	2.53	568.02	2.54	28.16
	12	.05	41.00	.87	178.12	.30	1.21
	13	.05	36.20	.80	158.08	.28	.82
	14	Alkaline	112.15	4.21	577.65	2.30	26.53

Attention is called to the fact that the potassium liberated from the New Jersey greensand is much greater than that recovered from the Maryland greensand. This is to be expected, since the former had an initial potassium content of 5.88 per cent, while the latter contained only 1.42 per cent of potassium, as shown in Table I. It will be seen that the largest amount of potassium was extracted from compost 3, containing the New Jersey greensand, and the second largest amount from compost 10, which is the corresponding mixture made with Maryland greensand. The fact that both of these composts have twice the amount of manure contained in No. 4, 7, 11, and 14 would indicate that

comparatively large amounts of organic matter favor sulphofication and the liberation of potassium under the conditions of this experiment. These results are not in accord with those reported by McLean (*11*), who, working with sulphur-floats-soil composts, came to the conclusion that a compost is more efficient in the producing of available phosphorus in the absence of large amounts of organic material.

In Table VII the total potassium present in each compost, the water-soluble potassium at the start, and the maximum water-soluble potassium present at any one time during the period of 23 weeks are computed on the basis of the initial weights of the composts.

TABLE VII.—*Total potassium made water-soluble (dry basis)*

Compost No.	Material added to 1,500 gm. greensand.	Total number grams potassium in compost.	Water-soluble potassium at start (percentage of total).	Maximum water-soluble potassium present.	
				Gm.	Percentage of total.
1	None	83.38	0.037	0.070	0.084
2	Sulphur 500 gm.	83.38	.055	.275	.330
3	Sulphur 500 gm.; manure 500 gm.	85.68	.832	15.28	17.83
4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	86.58	.408	7.87	9.10
5	Sulphur 500 gm.; soil 500 gm.	87.48	.088	.812	.928
6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O	87.48	.094	.812	.928
7	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	86.58	.494	9.46	10.93
8	None	20.97	.112	.070	.333
9	Sulphur 500 gm.	20.97	.243	.251	1.20
10	Sulphur 500 gm.; manure 500 gm.	23.27	3.22	9.62	41.34
11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	24.17	2.57	6.88	28.50
12	Sulphur 500 gm.; soil 500 gm.	25.07	.295	.389	1.55
13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $Al_2(SO_4)_3$ 0.18 H_2O; 0.02 per cent $FeSO_4$ 0.7 H_2O	25.07	.275	.310	1.24
14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; $CaCO_3$ 10 gm.	24.17	2.33	6.49	26.85

Reference to the last two columns of Table VII will show that, while the actual amount of soluble potassium which formed in the composts containing the Maryland greensand was much smaller than that which formed in the composts containing the New Jersey greensand, the percentage of the total potassium made water-soluble in the former was much greater than in the latter. One of the causes for this difference is to be found in Table II, which shows the mechanical analyses of the two greensands. The individual particles are much smaller in the Maryland than in the New Jersey greensand, thus exposing a much greater surface to the solvent action of the acids. Also, the glauconite particles of the

former were softer than those of the latter and seemed to be more soluble, as is shown by composts 1 and 8 in Table V. These figures show that although the New Jersey greensand contains more than four times as much potassium as the Maryland greensand, the amount of water-soluble potassium is the same.

In considering Table VII it is pertinent to ask to what extent the manure has contributed to the total amount of potassium recovered in the water extract. To answer this question Table VIII has been prepared upon the assumption that all the potassium in the manure was made soluble and was recovered in the water extract.

TABLE VIII.—*Relation of potassium content of the manure to the water-soluble potassium obtained*

Compost No.	Total soluble potassium obtained from compost.	Total potassium in manure.	Maximum amount of potassium from manure.[a]
	Gm.	*Gm.*	*Per cent.*
3	15.28	2.30	15.05
4	7.87	1.15	14.62
7	9.46	1.15	12.16
10	9.62	2.30	23.91
11	6.88	1.15	16.72
14	6.49	1.15	17.72

[a] The percentages in this column are based on the assumption that all the potassium in the manure was made water-soluble.

From the last column of Table VIII it will be seen that even on this basis it is possible in only one case to account for more than 17 per cent of the potassium as coming from the manure. It is evident, therefore, that from 80 to 90 per cent of the potassium found in the water extract must have come from the greensand or from the soil and greensand.

Referring again to the manure composts in Table VII, it will be seen that the total amount of potassium recovered by water extracts from these composts varies from 9.1 per cent to as much as 41.3 per cent of the total initial amount present.

It is important to consider the relation between the oxidation of sulphur and the liberation of potassium. This relation is a converging ratio, which was rather wide during the period of greatest oxidation of sulphur and diminished rapidly as the potassium was released. While it was not expected that this ratio would be resolved to a constant figure for all of the composts, because of the different materials used, in each series the composts containing manure do show a rather uniform relation between these processes. On the basis of the initial weights of the composts, Table IX shows the maximum number of grams of sulphur oxidized and of water-soluble potassium obtained, and their ratio, as determined from the water extracts.

TABLE IX.—*Relation between number of grams of sulphur oxidized and number of grams of potassium made water-soluble*

Compost No.	Sulphur oxidized.	Potassium made water-soluble.	Ratio of grams sulphur oxidized to grams water-soluble potassium.
	Gm.	*Gm*	
3	77.43	15.28	5.07:1
4	46.65	7.87	5.92:1
7	52.79	9.46	5.58:1
10	70.15	9.62	7.29:1
11	55.51	6.88	8.07:1
14	56.52	6.49	8.70:1

In the New Jersey greensand composts, approximately 5½ gm. of sulphur were oxidized for each gram of potassium made water soluble. For the Maryland greensand composts, the ratio is approximately 8 to 1. The ratio varies with the materials used, the high-potassium greensand having a lower ratio than the low-potassium greensand, and the composts containing 20 per cent manure having a lower ratio than those containing 10 per cent manure. For the composts in which soil was substituted for all the manure the figures are not shown, but the ratio is much wider, the amount of sulphur oxidized not being sufficient to make water soluble any large amount of potassium.

The results of this investigation would indicate that the composting of greensand, or of soil rich in postassium, with sulphur and manure may prove to be a practical and efficient method for obtaining available potassium from comparatively insoluble materials.

SUMMARY

Two greensands, one containing 5.88 per cent of potassium and the other 1.42 per cent, were used in studying the effect of sulphofication upon the solubility of the potassium. The outstanding results of the investigation are summarized in the following paragraphs.

(1) In composts consisting of greensand, manure, and soil in different proportions, an appreciable amount of the potassium of the greensand was made water-soluble through sulphofication.

(2) The composts containing the largest proportion of manure developed the highest degree of acidity, oxidized the greatest amount of sulphur, and produced the largest quantity of water-soluble potassium.

(3) The composts in which soil was substituted for a part of the manure developed less acidity, oxidized less sulphur, and produced a smaller amount of soluble potassium.

(4) When all the manure was replaced by soil, the rate of sulphofication was so slow that at the end of 23 weeks only a very small amount of acidity had developed and very little potassium had been made soluble.

(5) When no organic matter was added, the amounts of acidity and soluble sulphates were no greater than might be accounted for by the natural oxidation of the sulphur.

(6) The addition of small amounts of ferrous and aluminum sulphates failed to stimulate sulphofication.

(7) Calcium carbonate added to the sulphur-manure-soil compost produced a stimulating effect during the early part of the period but failed to increase the acidity, soluble sulphates, or potassium above the maximum reached by the corresponding compost in which no calcium carbonate was used.

(8) More water-soluble potassium was formed in the composts containing the high-potassium greensand, but a larger percentage of the total potassium present was liberated in the composts containing the low-potassium greensand.

(9) In the composts containing manure, the total amounts of potassium recovered in the water extracts varied from 9.1 per cent to a maximum of 41.3 per cent of the total initial amount present.

(10) Our results indicate that the composting of greensand, or of soil rich in potassium, with sulphur and manure may prove to be a practical and efficient method for obtaining available potassium from comparatively insoluble materials.

LITERATURE CITED

(1) Ames, J. W., and Boltz, G. E.
1919. EFFECT OF SULPHOFICATION AND NITRIFICATION ON POTASSIUM AND OTHER SOIL CONSTITUENTS. *In* Soil Sci., v. 7, no. 3, p. 183–195. References, p. 195.

(2) —— and Richmond, T. E.
1918. EFFECT OF SULPHOFICATION AND NITRIFICATION ON ROCK PHOSPHATE. *In* Soil Sci., v. 6, no. 5, p. 351–364. References, p. 364.

(3) Blair, A. W.
1916. THE AGRICULTURAL VALUE OF GREENSAND MARL. N. J. Agr. Exp. Sta. Circ. 61, 13 p., 1 fig.

(4) Brown, P. E., and Gwinn, A. R.
1917. EFFECT OF SULPHUR AND MANURE ON AVAILABILITY OF ROCK PHOSPHATE IN SOIL. Iowa Agr. Exp. Sta. Research Bul. 43, p. 369–389, 4 fig. Bibliography, p. 389.

(5) —— and Warner, H. W.
1917. THE PRODUCTION OF AVAILABLE PHOSPHORUS FROM ROCK PHOSPHATE BY COMPOSTING WITH SULPHUR AND MANURE. *In* Soil Sci., v. 4, no. 4, p. 269–282, 3 fig. References, p. 282.

(6) Cook, George H.
1868. GEOLOGY OF NEW JERSEY . . . xxiv, 899 p., illus., 1 fold. col. map. Newark, N. J.

(7) Hilgard, E. W.
1906. SOILS, THEIR FORMATION, PROPERTIES, COMPOSITION, AND RELATIONS TO CLIMATE AND PLANT GROWTH IN THE HUMID AND ARID REGIONS. xxvii, 593 p., illus. New York, London.

(8) LIPMAN, Jacob G., and BLAIR, A. W.
1918. VEGETATION EXPERIMENTS ON THE AVAILABILITY OF PHOSPHORUS AND POTASSIUM COMPOUNDS. *In* N. J. Agr. Exp. Sta. 38th Ann. Rpt. [1916]/17, p. 353-368.

(9) ——— and McLEAN, Harry C.
1918. EXPERIMENTS WITH SULPHUR-PHOSPHATE COMPOSTS CONDUCTED UNDER FIELD CONDITIONS. *In* Soil Sci., v. 5, no. 3, p. 243-250.

(10) ——— ——— and LINT, H. Clay.
1916. SULPHUR OXIDATION IN SOILS AND ITS EFFECT ON THE AVAILABILITY OF MINERAL PHOSPHATES. *In* Soil Sci., v. 2, no. 6, p. 499-538, 5 fig. Literature cited, p. 535-538.

(11) McLEAN, Harry C.
1918. THE OXIDATION OF SULFUR BY MICROÖRGANISMS IN ITS RELATION TO THE AVAILABILITY OF PHOSPHATES. *In* Soil Sci., v. 5, no. 4, p. 251-290. References, p. 287-290.

(12) PATTERSON, H. J.
1906. RESULTS OF EXPERIMENTS ON THE LIMING OF SOILS. Md. Agr. Exp. Sta. Bul. 110, 56 p.

(13) TRUE, Rodney, and GEISE, Fred W.
1918. EXPERIMENTS ON THE VALUE OF GREENSAND AS A SOURCE OF POTASSIUM FOR PLANT CULTURE. *In* Jour. Agr. Research, v. 15, no. 9, p. 483-492, pl. 33-34.

RUST IN SEED WHEAT AND ITS RELATION TO SEEDLING INFECTION[1]

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INTRODUCTION

The fact that the mycelium of rust fungi in some cases may enter the seed and seed parts of various plants and produce spore bodies there has been known for many years and has been referred to by various writers. Differences of opinion have existed, however, as to the importance of this phenomenon in the dissemination of the rust concerned. Aside from the occurrence of rust in and upon these plant organs, other facts have seemed to indicate that rust might be transmitted by means of seed. A number of cases are on record where the uredinial and telial stages of various rusts have suddenly appeared in regions where the aecial host was unknown. Lagerheim (*17*)[2] found *Puccinia coronata* Cda. on oats in Ecuador, and since no species of Rhamnus known to bear the aecia of this rust occur there he concluded that the rust was probably introduced by means of oats brought from Europe. He also reported stemrust doing great damage in Ecuador, although barberry bushes were not present there. According to McAlpine (*18, p. 60*), *P. graminis* is common in Australia, while only a very few hedges of barberry exist and the aecial stage of this rust has never been found occurring naturally upon that continent. Bolley and Pritchard (*5, p. 647*) quote McAlpine as saying that he is convinced that certain grass seeds secured by him from the United States Department of Agriculture introduced certain rusts into Australia. Among these he named *P. coronata* Cda. on the grass *Beckmannia erucaeformis* and *P. montanensis* Ell. on wild rye (*Elymus canadensis*). Numerous other similar instances could be cited.

The widespread occurrence of rust epidemics has not been satisfactorily explained, to some pathologists at least, by our present knowledge of the overwintering of the uredinial stage or by our present knowledge of the importance of infection of wheat by aeciospores from the barberry. These conditions have caused a number of writers to attempt to explain sporadic attacks of rust by a theory of seed transmission. The idea is

[1] The investigations reported in this paper were carried on at Madison, Wis., under the direction of the Office of Cereal Investigations, United States Department of Agriculture, Washington, D. C. The writer wishes especially to thank Dr. L. R. Jones and Dr. A. G. Johnson, of the Department of Plant Pathology of the University of Wisconsin, and Dr. H. B. Humphrey, of the Office of Cereal Investigations, United States Department of Agriculture, for helpful suggestions and criticisms during the progress of the work and in the preparation of the manuscript.

[2] Reference is made by number (italic) to "Literature cited," p. 275-277.

not new that certain fungous parasites may exist in the vegetative state in the seeds of their hosts and be thus transmitted from one generation to another. Even before this was established for certain of the cereal smuts, various workers had endeavored to show this condition for the cereal rusts. The discovery that certain smuts were systemic in their infection gave impetus to further research along this line.

The purpose of the investigations reported here was to determine whether or not *Puccinia graminis tritici* Erkiss. and Henn. can be transmitted to the seedling by being carried over with the seed grain.

OCCURRENCE OF RUST IN SEEDS AND SEED PARTS OF VARIOUS PLANTS

The earliest report that the writer has been able to find that stemrust may attack the seed and seed parts of grain was made by W. G. Smith in 1885 (*23*). He found telia of *Puccinia graminis* in the pericarp of oat kernels and figured teliospores within the oat grains lying inside the aleurone layer and between that and the endosperm. His drawings and notes, however, leave much to be desired. In 1886 (*24*) the same author figured aecia embedded in the fruits of the barberry. Maddox (*18, p. 20*) noted rust infection upon—

> the young half-grown grain . . . before it had started to go out of the milk stage.

He does not state to which rust he refers. Pritchard (*22, p. 151*) in 1911 figured stemrust upon wheat kernels and stated that telia and fragments of mycelium were found in abundance in the pericarp of wheat kernels and that seed infection occurs very frequently even in rust-free years. Other reports have been made of *P. graminis* upon the caryopses of wheat, oats, barley, and various grasses, and the writer has observed this condition upon all of the above-mentioned hosts.

Puccinia glumarum (Schm.) Erikss. and Henn. is also known to occur commonly upon the caryopses of wild and cultivated Gramineae. Beauverie (*1, 2*) has recently reported at length upon this phenomenon and states that if the seed is hulled the sori are produced upon the interior of the glumule, while if the seed is naked they are formed in the pericarp. He found this rust more or less abundant in the caryopses of *Triticum vulgare*, *Hordeum vulgare*, *Brachypodium pinnatum*, *Agropyron caninum*, and *Bromus mollis*. He also reports finding *P. simplex* on barley kernels and *P. coronata agropyri* [1] on *Agropyron repens*. Blaringhem (*3, p. 86*) found somewhat the same conditions reported by Beauverie. Eriksson and Henning (*10, p. 199, pl. 7, 9*)) fully describe and give excellent figures of whole kernels and cross sections of kernels infected with *P. glumarum*.[2] Various other rusts have been reported as occurring upon seeds and seed parts of various plants. Carleton (*6, p. 28–29*) has reported the occur-

[1] It is not clear what rust is referred to by this name.

[2] These authors cite several former observations of *P. glumarum* upon kernels of wheat, the earliest of which was by Schmidt (*10, p. 454*) in 1819.

rence of Euphorbia rust (*Uromyces euphorbiae* C. and P.) upon seeds of *Euphorbia dentata*. Various writers have noted *P. malvacearum* Mont. on hollyhock seeds. Other examples of a similar nature could be given. The discussion of the practical importance of this occurrence in relation to subsequent infection of seedlings will be taken up in a later paragraph.

ABUNDANCE OF KERNEL INFECTION IN WHEAT

In order to learn to what extent seed wheat may become infected with *Puccinia graminis tritici* a large number of wheat samples were examined by the writer. These samples were secured from various sources and from the crops of the two years 1915 and 1916. During the fall and winter of 1915–16 samples of wheat were secured from various points in North and South Dakota, from western Minnesota, from grain commission firms in Minneapolis, and from wheat grown in the rust nursery at the University Farm, St. Paul, Minn. In all, several hundred samples of wheat were examined, all of which came from fields known to be badly rusted or from localities where it was known that rust epidemics had occurred. During the fall of 1916 a large number of samples of wheat were obtained from the same regions as in the previous year. In those regions there occurred that year an unusually severe rust epidemic. It would seem, therefore, that under these conditions there would be as large an amount of seed infection as ever occurs.

It was found at once that the task of determining the percentage of infection was not so easy as it at first appeared. In some cases the kernels were found to be but slightly infected, having only one sorus on the hilum, or germ end. In such cases it was impossible to see that these were infected at all except by means of examination under considerable magnification. In other cases the general appearance of the kernels seemed to indicate to the unaided eye that there was rust infection, but upon examination under the microscope no rust was found. Indications were that such discolorations were caused by some other agency. Alternaria and Helminthosporium species were often found to be associated. In general, it was found impossible to tell in every case whether or not a kernel was infected by rust except by microscopic examination. However, in many cases, especially after some experience, many of the rust-infected kernels could be easily detected by the unaided eye.

The large majority of the rust-infected kernels, when mature, were found to bear only telia,[1] which appeared as glistening black specks on the hilum, or the germ end, or a short distance down the groove of the kernel. Sometimes sori were noted a short distance from the hilum with no surface connections between these and the ones at the hilum (see Pl. 39, B). Upon sectioning similar kernels, however, the mycelial connections were found. If the hilar end of an infected kernel is scraped with a sharp knife or scalpel, teliospores in abundance can be secured.

[1] Uredinia were noted on immature kernels at various times.

In order to learn the percentage of infection and also to be absolutely certain that all seed used in experimental work was infected with rust, the following method of selecting rusted kernels was employed. The samples of wheat to be examined were spread out in a shallow dish where the light was good, and the discolored kernels were taken out one by one by means of small forceps. A common 5-inch reading glass usually was used to facilitate making the selections. These discolored kernels were then placed one by one under a low-power binocular microscope where it could be easily determined at a glance if any rust sori occurred on their germ ends. As will be shown later, some infected kernels may have been missed, for sometimes the sori on the germ ends are broken off with the flowering glumes in thrashing.

Bolley and Pritchard (*5, p. 646*) state that—

in some samples of wheat in the rust-infected crop of 1904 as high as 30 per cent of all grains harvested showed such rust infection.

Pritchard (*22, p. 153*) also states that in 1910, a rust-free year, wheat from elevators at Brookings, S. Dak., showed some rusted kernels in every sample and many in some varieties, especially Bluestem. The writer's observations do not agree with this. In all the hundreds of samples examined the largest percentage of kernels found in any one sample showing rust sori was only about 1 per cent of the total. Many samples were examined in which no infected kernels could be found. In fact, even in 1916, a very bad rust year, the varieties having kernel infection were the exception rather than the rule. Moreover, varieties of the durum wheat were the ones which most often were found to be infected. This was the case in both years and seems to be consistently so. One sample of mixed wheat from Reeder, N. Dak., collected in 1916, contained about 1 per cent of infected kernels. Although the sample contained Marquis, durum, and Bluestem in the mixture, only durum kernels were found infected. This has been found to be the case in many mixed samples examined. Only in a few cases have any number of infected kernels of other varieties been found. This may be due to the fact that the spike of durum wheat is so compact that it dries very slowly after rains or heavy dews and these moist conditions favor infection by rust. It is a well-known fact that durum varieties are very susceptible to Fusarium scab, possibly for the same reason.

To illustrate this point the following observation is of interest. The writer noted in 1916 at Dickinson, N. Dak., that all the durum wheats were more or less badly rusted on the heads. (See Pl. 38.) This was especially true of the Kubanka strain known as selection No. 8, C. I. No. 4063 (Pl. 38). A large number of heads of this variety were collected which were literally covered with stemrust sori (Pl. 39, A). Mr. Ralph Smith, of the Office of Cereal Investigations, stationed at Dickinson, kindly furnished the writer some of the seed of this variety when the plots were thrashed. This seed was all examined carefully, and it was

found that only about one kernel in a thousand showed any evidence of rust infection.

METHOD OF KERNEL INFECTION

There are two possible methods by which kernel infection takes place. First, the kernel itself may become infected by urediniospores lodging upon its surface under the glumes; or, second, the infection may spread from sori produced upon the inclosing glumes, upon the rachis or the rachilla. Since there probably are no stomatal openings upon the kernel itself and since uredinial infection takes place only through the stomata, the first possibility seems to be eliminated. Cobb (7) reports finding urediniospores of stemrust in abundance in the brush of the kernel of a large number of varieties of wheat, even after the wheat was thoroughly cleaned. He also reports finding stomata near the brush end and concludes that infection of the kernel may take place at this point. He found sori common on wheat kernels but does not say anything with regard to their location.

The writer has never found sori of stemrust produced near the brush end of wheat kernels nor has he been able to find stomata upon wheat kernels at any time in their development. As previously stated the writer has found rust sori on wheat kernels at or near the germ end. This would indicate that infection takes place by the spread of rust mycelium to the caryopsis from infection which had previously taken place at the base of the glumes or on the rachilla. Indeed, our experiments have confirmed this. When kernels were examined in the wheat head and were found to be infected, it was found that one or more of the flowering glumes always bore sori; and frequently several sori on the rachis, rachilla, and glumes were found to be confluent and extending over to the base of the kernel. The tissue of the hilar region of the kernel is similar in its structure to leaf tissue, and therefore infection of this region might be expected. In samples of thrashed grain kernels with adhering pieces of glumes often had rust sori extending from the base of the glume to the kernel itself. The glumes seemed to be held thus by the fungus (Pl. 39, B).

That infection may spread from the glumes to the kernel hilum was shown as a result of artificial inoculation experiments. These were carried out as follows. Artificial inoculations of wheat heads with urediniospores of stemrust were made in the greenhouse during the winter of 1915–16. The first set of inoculations was made when the kernels were less than half grown. Urediniospores were dusted in abundance inside the glumes, and the heads were sprayed with distilled water, inclosed in large test tubes, and kept for two days. Wet cotton was kept in the bottoms of the tubes and the mouths were plugged with cotton, thus giving the conditions necessary for infection. The first attempt was a failure, either because too many spores were used or

because the kernels were not developed far enough to survive the invasion of the parasite, and the infection was so great that none of the kernels developed. The glumes and rachis at the base of the spikelet in each case were covered with sori 10 days after inoculation and the inner surfaces of the glumes were filled with urediniospores.

These results appear to confirm Johnson's (*15*) observations regarding the effect of rust infection upon floret sterility in wheat. He found floret sterility increased 20.03 per cent when wheat heads were sprayed with a water suspension of a mixture of urediniospores of *Puccinia graminis* and *P. triticina*. His conclusions were that when the rust attacks the ovary early enough it prevents its development, and other semiparasitic fungi complete the process of destruction, while if it attacks the embryo after it is fertilized and has begun to enlarge, a rusted kernel results. Table I shows the outcome of a second set of inoculations. Kubanka wheat (C. I. No. 1440) was used for these experiments. The glumes were opened, and a very few spores were placed at the base of the inside of the glumes with a fine platinum needle. The heads were then sprayed with distilled water and inclosed in a test tube as before. Every spikelet in each head, with the exception of the smallest ones at the tip, was thus inoculated.

TABLE I.—*Results of artificial inoculation of wheat ovaries at different stages of development*

Host No.	Condition of ovaries.	Date of inoculation.	Number of heads inoculated.	Date thrashed.	Number of infected kernels.	Number of healthy kernels.
5	Ovaries two-thirds grown.	Nov. 15, 1915	2	Jan. 11, 1916	3	8
6	.....do.	Nov. 18, 1915	1	.....do.	2	1
7	Ovaries size of pinhead.	.....do.	1	.....do.	0	0
8	.....do.	.....do.	1	.....do.	0	1
9	Ovaries somewhat larger than above.	.....do.	10	.....do.	0	2
10	Kernels two-thirds grown.	Nov. 15, 1915	4	.....do.	3	5
11	.....do.	Dec. 5, 1915	3	.....do.	15	8

It will be noted from Table I that in no case was kernel infection obtained when inoculations were made while the ovary was very small. On the other hand, when the inoculations were delayed until the kernels had attained about two-thirds of their normal size at maturity, the kernels were able to continue development, and a high percentage of rusted ones resulted. It would seem, therefore, that the amount of kernel infection each year does not depend alone upon the amount of rust occurring upon the heads of the wheat but also upon the time when this infection takes place and whether the kernels are at the right stage of development to become infected. The weather conditions where the kernels are at the right stage of development are also a very important factor.

EFFECT OF KERNEL INFECTION UPON GERMINATION

Large numbers of rust-infected wheat kernels were germinated and grown to various stages of development for the purpose of making histological studies. Parallel series of unrusted kernels from the same seed lot were germinated and grown for comparison. In these series it was noted that the rusted and unrusted seed gave practically identical percentages of germination.

RUST TRANSMISSION WITH SEED GRAIN

HISTORICAL DISCUSSION

From the vast amount of work which has been done upon this problem it is possible to separate three main theories. Briefly stated, these theories are as follows: (1) Mycoplasm theory of Eriksson; (2) dormant mycelium in the seed carrying infection to the seedling; and (3) seed-borne spores causing infection of the seedling.

MYCOPLASM THEORY OF ERIKSSON

Eriksson (*9*) in 1897 announced his well-known mycoplasm theory. He states that in the summer of 1893, upon microscopical examinations of sections of very young sori of yellow-rust (*Puccinia glumarum*) upon wheat leaves, he found adjacent to these sori, besides the usual cell elements, peculiar, elongated, mostly faintly curved, plasmatic corpuscles. He concluded (*p. 193*, translation) that—

> these plasma corpuscles, at first freely swimming in the protoplasm, constitute a phase of the fungus, the primary phase, wherein the fungus by its independent appearance makes itself visible. The fungus has for weeks, months, possibly even years, previously led a latent existence in an invisible form and alongside the protoplasm of the host plant, forming a kind of mycoplasm-symbiosis between host and parasite.

Although Eriksson describes this mycoplasm in detail and figures it in various stages of development, very few later writers have accepted his evidence as being in any way conclusive. While it is not the present purpose to give a detailed criticism of the theory, yet, in the judgment of the writer, it seems that Eriksson's experimental evidence does not establish his contention in regard to the existence of the so-called mycoplasm. Nothing similar has been encountered in any of the hundreds of sections which the writer has made. More will be said later of this experimental evidence upon which Eriksson based his conclusions. Ward (*25, p. 353*) sums the matter up very well when he states that Eriksson merely—

> inverts all the stages of the fungous attack on the cell, and supposes the last stage to be the first and that this error and misrepresentation of the microscopic appearance account for the whole wearisome persistence in an inherently improbable hypothesis.

Detailed criticisms of Eriksson's theory are given by Bolley (*4*), Zukal (*26*), Ward (*25*), and Massee (*20*). Others could be added to this list,

but it is sufficient to say that no pathologist of note has for any length of time accepted this explanation of rust dissemination.

DORMANT MYCELIUM THEORY

There has been more support, and probably more ground for support, for the theory that the mycelium of rusts may live over in the seed or seed parts of the plant in a dormant state and then infect the young seedlings at the time of germination. A number of writers have suggested this possibility, among whom W. G. Smith (*24*) was probably the first. He says:

> If apparently healthy leaves of corn are taken, and apparently healthy leaves of Barberry, and these leaves are microscopically examined, fungus mycelium will be commonly found inside the leaves. Neither is the mycelium confined to the leaves, for it invades the seeds of both plants, and these seeds are frequently planted with the mycelium in their tissues. A diseased progeny is the result.

Zukal (*26*), in 1899, published observations which seemed to indicate to him that rust was transmitted by mycelium in seed grain. He concluded that rust mycelium might live over in the wheat kernels because the rust appeared so early on the young seedlings. He found septate mycelium at the base of the sheath, in the culms, and at the nodes in the parenchyma cells just under the epidermis. He concluded that the mycelium lived over in the seed and in the spring grew through the scutellum into the embryo and developed with the plant.

Pritchard (*22, p. 152*), in 1911, found mycelium in the roots, in both central cylinder and epidermis, in the stem, and between the leaf sheaths in plants grown from rusted wheat kernels. This mycelium resembled rust mycelium which he found at the base of the sori upon the germ end of the kernel of wheat from which the plants were grown. He states that the mycelium was abundant in the young stem, filling the intercellular spaces and freely penetiating cell walls as well. More will be said later in regard to Pritchard's work.

SEED-BORNE SPORES THEORY

Massee (*20*) secured evidence which seemed to indicate to him that seed-borne urediniospores or urediniospores in the soil might cause infection of young wheat plants. More recently Blaringhem (*3*) and Beauverie (*1*) have published extensive observations which they have made. They conclude that *Puccinia glumarum* may be transmitted by urediniospores borne in the pericarp of the seed. As stated above, they found uredinia in abundance in the pericarp of various grains and grasses and concluded that these spores, so protected, may retain their viability until the germination of the seed, when they become free from the sori through the rupturing of the pericarp and may infect the young plant at this time. Their conclusions, in the writer's judgment, are based upon insufficient experimental evidence, and, although the theory is interesting in itself, certainly it should be supported by more careful experiments.

EXPERIMENTS OF VARIOUS WORKERS

A number of workers have grown plants from rusted seeds of various kinds under various degrees of isolation. The results of these experiments are rather variable. The writer has assembled the results and the methods used in several of these experiments in Table II, which includes the experiments of nine men conducted at different times in different countries. None of these writers claimed to have secured normal conditions for the growth of the host plants, and in no case was any record taken of the atmospheric conditions inside the devices used to secure isolation.

TABLE II.—*Summary of results obtained by other investigators in experiments on seed transmission of rusts*

Experimenter.	Year.	Place of experiment.	Rust involved.	Means of isolation.	Kind of seed used.	Results.
Eriksson (*9*)	1892–1898	Sweden	*P. glumarum, P. graminis.*	Ventilated glass frames.	Barley, wheat	Few positive.
Klebahn (*16*)	1899	Germany	*P. graminis, P. glumarum.*	Glass cages	do	Uncertain.
Zukal (*26*)	1898	Austria	*P. glumarum*	Isolated garden	Wheat	Negative.
Linhart[a]	1898	do	do	Glass inclosures	do	Do.
Hayman (*12*)	1903–1907	India	*P. glumarum, P. triticina.*	Glass cages	do	Uncertain.
Bolley (*4*)	1905	North Dakota.	*P. graminis*	do	do	Negative.
Massee (*20*)	1894	England	*P. glumarum*	Bell jars	do	Positive.
Nowikoff[b]		Russia	*P. coronata, P. glumarum.*	Isolated cages	Oats, barley	Negative.
Jaczewski (*14*)	1902–1906	do	do	Glass cages	Oats, rye	Do.

[a] Reference is made to Linhart's work by Zukal (*26*); original work not published.
[b] Referred to by Jaczewski (*14*); original not seen.

Eriksson carried on experiments for seven years and secured only a very few infections upon plants grown inside his "isolation frame." This frame was made of glass with wooden corner posts and an iron roof. Ventilation was secured by drawing air through a cotton filter. At best a cotton filter is not very satisfactory, and it is to be noted that Eriksson secured his positive results after the cages had been used three or four years. Grove (*11, p. 45–47*) makes an interesting comment upon Eriksson's work. He says (*p. 45*)—

on some of his "protected" plants aphides also made their appearance, yet this does not seem to have suggested to him [Eriksson] that the *zooplasm* of the aphides must also have been latent in the seed. If the aphides got in, so would fungus spores, since it has been proved that uredospores are carried by them and other insects.

Klebahn repeated Eriksson's experiments and found one plant infected with *Puccinia graminis* in his glass cages. He explains this (*16*) by the fact that this infection did not appear until a few days after he had been working with *P. graminis* near this cage. The time which had elapsed was about the normal incubation period for this rust. It seems very likely, therefore, that the one infection noted originated from spores accidentally introduced.

Hayman (*12*) repeated his experiments for five years and grew 195 plants to maturity. The conditions inside his cages were abnormal at all times, although an effort was made to control conditions by means of a blacksmith bellows and cotton filters. Two pustules of rust appeared in the fifth year, but the author himself was not satisfied with this result as is evidenced by the fact that he states that the tar used to coat the inside of the cages had oozed through the cracks in the cage in which the plant was found to be infected.

Massee, the only other worker who secured positive results, used bell jars placed upon cotton wool with a cotton plug in the opening at the top. He sowed wheat inside these jars, which was known to be shriveled by *Puccinia glumarum*, and as controls he sowed plump seed of the same variety. Sixty per cent of the infected seed germinated, and when the plants were 3 inches high rust appeared in each pot. When the plants were 5 inches high 26 per cent of them were rusted. Of the plump seed sown under the same conditions 96 per cent germinated, and all remained perfectly free from rust. These results are striking, and the problem with this rust is highly deserving of further investigation.

Pritchard (*21*) grew 60 wheat plants from rusted seed in glass cages in the open and later repeated the experiment in the greenhouse. No rust appeared on any of the plants. He states that the plants headed and blossomed but no kernels developed because the temperature and moisture conditions were abnormal. He also refers to an experiment where wheat sown at different dates was inoculated with both aeciospores and urediniospores of stemrust. Rust did not appear abundantly, however, until the wheat began to head, when each sowing became thoroughly rusted. He states that it is possible to attribute this peculiar behavior to infection through the seed with a long subsequent incubation period in the growing plant. It seems to the writer that this conclusion is entirely unwarranted, since it is well known that infection with stemrust is much more easily obtained and more noticeable during the heading period of the plant when stemrust does such great damage by attacking the neck of the stalk. This is a period of rapid growth of the plant and a period when urediniospores are usually present in abundance in the air. If climatological conditions are favorable—that is, if high relative humidity and comparatively low temperatures prevail during this period—a severe rust epidemic is almost sure to follow if the infection material is present. A study of the climatological conditions during the last of June and the first of July in the spring-wheat belt shows that these conditions existed in the years when rust epidemics were severe and did not exist in the years when rust was not prevalent. These conditions are sufficient to explain any such peculiar behavior as Pritchard refers to and also help to explain rust epidemics in the spring-wheat region.

EXPERIMENTAL DATA

It is seen from the foregoing review that a number of workers have grown rust-infected seed grain under various degrees of isolation and with more or less conflicting results. The evidence of this kind as to the transmission of *Puccinia graminis* by means of seed seems to be largely negative. Nevertheless some positive results have been reported. The one conclusive way to prove the contention that rust can be carried on seed grain must be to produce the disease upon plants grown under controlled conditions from seed known to be infected with the rust. While histological evidence is valuable from the standpoint of interpretation, yet no amount of such work by itself is fully convincing in connecting seed infection with the appearance of the disease upon the leaves unless plants can be grown from infected seed under controlled conditions and the disease be produced upon these plants. The writer's experimental investigations were along three lines: (1) Greenhouse experiments in which rusted kernels of wheat in large numbers were sown under isolated conditions and the resulting plants watched for infection; (2) field experiments in which rusted wheat kernels were sown in the fields and watched to learn if infection occurred upon the resulting plants sooner than upon plants grown from clean seed; and (3) histological investigations in which rusted wheat kernels were germinated under various conditions and the resulting seedlings examined histologically for spread of rust infection from the kernel to the seedling.

GREENHOUSE EXPERIMENTS

The writer determined to test this matter thoroughly by growing a large number of wheat plants from kernels known to bear sori of stemrust, under conditions of isolation and at the same time under conditions normal for the development of the host. In order to meet these requirements a room in the pathological greenhouses at the University of Wisconsin was equipped as shown in Plate 40. The room was examined carefully and every crack and opening sealed. Double doors were constructed with a space between, which could be sprayed each time before the room was entered. An adjustable shade was placed upon the roof in such a way that a spray of water could be thrown upon the glass underneath the shade to aid in cooling the room, and a system of forced circulation of washed air was installed, as shown in figure 1. Thermograph and hygrograph records were kept at all times when the plants were growing, and it was found easily possible to control the temperature and humidity within normal limits for growth of wheat plants. Plants grown in this house were entirely normal in appearance and produced plump kernels in every head. The accompanying photographs (Pl. 41) taken at different times during the period when the experiments were in progress, show the normal, healthy condition of the plants. In order to test the efficacy of this air-washing apparatus, about a pint

of smut spores were thrown up into the opening at *i* in figure 1, and an attempt was made to catch any which went through the drum upon moistened sterile cotton held at *h*. This cotton was then washed and the water carefully examined with the microscope. No spores could be found upon this cotton, although the experiment was repeated several times. Every time the room was entered the space between the double doors was thoroughly sprayed, and a rubber coat which was kept hanging in this antechamber was put on. Although wheat was grown in the adjacent houses and became badly infected with mildew (*Erysiphe graminis*) none appeared on that grown inside of the isolated room. Neither did any aphids, which were plentiful at various times in other rooms in the greenhouse, make their way into the isolated room.

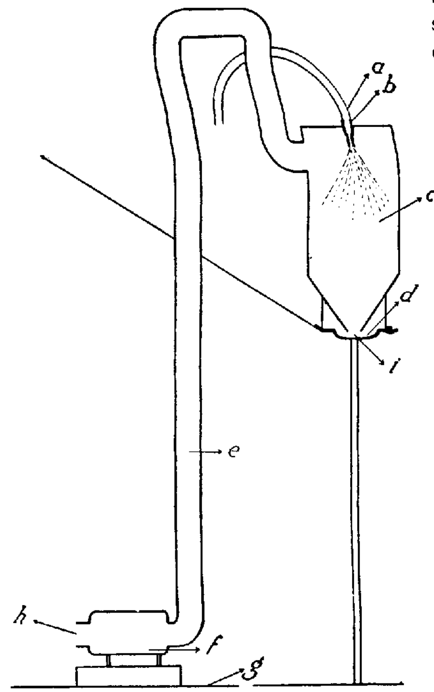


FIG. 1.—Diagram of air-washing apparatus for isolated room used for growing rust-infected seed: (*a*) Hose connection; (*b*) spray nozzle; (*c*) galvanized iron cylinder; (*d*) greenhouse gutter into which the water from spray drained; (*e*) connection pipe from cylinder to blower; (*f*) electric blower; (*g*) floor of greenhouse; (*h*) mouth of the blower where air entered the room; (*i*) air intake.

The soil used in these experiments was in every case sterilized, and only boiled water was used for watering until after the lake from which the water supply was derived was frozen over.

Four different lots of rusted seed were grown at different times in this house. Each lot was sown in flats 12 inches wide, 24 inches long, and 6 inches deep. These experiments will now be considered in the order in which they were performed.

EXPERIMENT 1.—Seed for this experiment was selected from lots of wheat obtained from the following sources: Four varieties of durum from the cereal-disease plots at Madison, Wis.; one lot of Marquis from Maynard, Iowa; one mixed lot of wheat from Leith, N. Dak.; one lot of durum from Brookings, S. Dak.; one mixed lot of unknown source from a

grain elevator in Minneapolis, Minn.; one lot of durum from Hagen, N. Dak.; and one mixed lot from Fargo, N. Dak. From all of these wheats rusted seed was selected and sown on November 8, 1915, in the isolated room. Seven hundred and six plants were obtained from this seed and grown to maturity. No rust appeared upon any of the plants at any time. On the primary leaf of two different plants lesions appeared from which cultures of *Helminthosporium* sp. were obtained. No other infection of any kind appeared upon any of these plants. Plate 41, B, shows three flats of plants from this experiment just after the plants were well headed.

EXPERIMENT 2.—Experiment 1 was carried on during the winter months, and it was thought advisable, therefore, to duplicate the work in the spring and sow the seed at the time spring wheat normally would be sown in the field. The same precautions were taken as in experiment 1, and the same room was used. Seed was secured from the following sources: Three lots of mixed seed of unknown source from Minneapolis, Minn.; two lots of mixed seed of unknown origin from Minneapolis, Minn.; three lots of durum from the rust nursery, University Farm, St. Paul, Minn.; one lot of durum from Clark, S. Dak.; two lots of durum from the cereal-disease plots at Madison, Wis.; one lot of Marquis from Maynard, Iowa; one lot of durum from Leith, S. Dak.; one lot of mixed seed from Armour, S. Dak. Rusted kernels from these sources were sown on March 19, 1916, and 730 plants emerged and were grown to maturity. No rust appeared on any of these plants at any time. The experiment was discontinued when the wheat became mature.

EXPERIMENT 3.—The experiment was repeated during the winter of 1916–17, when 760 plants were grown to maturity under the same conditions as outlined above. Seed for this experiment was obtained from various places in North and South Dakota and Minnesota. No rust appeared upon these plants at any time. The experiment was concluded when the plants were mature.

EXPERIMENT 4.—It was thought possible that soil temperatures at the time of the germination of the seed might affect the ability of the fungus to penetrate the young embryo and that the temperature in the isolated room might have been too high for successful infection at the time of germination. In order to simulate more closely natural conditions of germination and growth of the plants, infected wheat kernels were germinated in soil in an Altmann incubator at different temperatures as indicated in Table III.

When the seedlings were about 1½ inches long, they were carefully transferred to pots of sterilized soil and grown in the isolated room until the plants were mature. Twenty-five kernels of wheat were used for each temperature indicated. No rust appeared upon these plants at any time.

TABLE III.—*Temperatures at which infected wheat kernels were germinated*

Date of germination.	Number of plants.	Temperatures.	Date of transfer to greenhouse.
Dec. 12, 1916	21	−2° C. alternated with 15° C.	Dec. 30, 1916.
Do.	20	7° C. alternated with 15° C.	Dec. 26, 1916.
Do.	20	12° C. continuously	Do.
Do.	23	2° C. alternated with 21° C.	Do.
Do.	24	10° C. alternated with 18° C.	Do.
Do.	24	15° C. continuously	Do.

EXPERIMENT 5.—A number of writers have suggested the possibility of rust infection taking place from spores on the surface of the seed. To test this possibility, several flats of wheat were sown with seed that had been literally covered with viable urediniospores of stemrust. Preston wheat (C. I. No. 3081) was used for this experiment. In all, about 200 plants were grown. No rust infection appeared upon any of them at any time.

FIELD EXPERIMENTS

EXPERIMENT 1.—In the spring of 1916 rusted wheat from various sources was sown in the field along with clean seed and rusted seed treated with the modified hot-water treatment. These plots were examined every few days from the first appearance of rust infection. After June 27 the plants were examined every other day. Table IV gives the methods employed and results obtained in the experiment.

The groups of plots numbered 1 to 4, 5 to 9, 10 to 13, and 14 to 18 were grown in different locations on the University Farm at Madison, Wis.

Stemrust was noted upon *Hordeum jubatum* near two of the plots on July 3, 12 days before it appeared upon the wheat in these plots.[1] Infection also had been common upon adjacent barberries for some time previously. It will be noted that the plants grown from badly rusted samples of seed did not develop rust any earlier or any more severely than those grown from clean seed or from rusted seed which had been treated with the modified hot-water treatment.

Recently the writer has had opportunity to consult the notes on an unpublished experiment somewhat similar to field experiment No. 1, as described above. The work was done by E. C. Johnson, at that time Pathologist in Charge of Cereal Disease Investigations in the Bureau of Plant Industry, and carried on at the University Farm, St. Paul, Minn., in 1912. The experiment is described and results are given in Mr. Johnson's report, a copy of which is on file in the Office of Cereal Investigations, Department of Agriculture, Washington, D. C.

[1] By inoculating wheat plants in the greenhouse this was found to be *Puccinia graminis tritici*.

TABLE IV.—*Development of rusts on plants grown in the field from treated and untreated rust-infected seed and rust-free seed in 1916*

Plot No.	Description of seed.	Date sown.	Treatment of seed.	Size of plots.	Date on which plants emerged.	Date on which plants headed.	Date of appearance of— Leaf-rust.	Date of appearance of— Stem-rust.	Abundance of infection by— Leafrust. Date.	Abundance of infection by— Leafrust. Percentage.	Abundance of infection by— Stemrust. Date.	Abundance of infection by— Stemrust. Percentage.
1	Marquis, many rusted pieces of glumes, some rust-tipped seed.	May 15	Untreated.	5 rows 10 feet long	May 20	July 14	June 10	July 14	June 16	1+	July 25	5+
2	do	do	Treated	do	do	do	do	do	do	1+	do	5+
3	Durum wheat, 1 per cent infection of kernels	do	Untreated	do	do	do	June 12	July 18	do	1—	do	Trace.
4	do	do	Treated	do	do	do	June 10	July 15	do	1	do	5—
5	Same seed as No. 1	May 23	Untreated	5 rows 1 rod long	May 28	July 20	June 12	do	June 19	2	do	3
6	Marquis (C. I. No. 3641), rust-free seed	do	do	do	do	do	do	July 14	do	2	do	5
7	Mixed seed from very badly rusted field, 1 per cent seed infection.	do	do	do	do	do	do	July 15	do	15	do	10
8	do	do	do	do	do	do	do	do	do	15	do	10
9	Preston (C. I. No. 3081), rust-free seed	do	do	do	do	July 22	June 14	do	do	5	do	6
10	Same as No. 7	May 3	do	6 feet by 15 rods	May 9	July 8	June 6	do	June 16	1	do	25
11	do	do	Treated	do	do	July 9	do	July 14	do	1	do	20
12	Durum seed, 1 per cent infection	do	Untreated	do	do	July 8	do	July 15	do	1	do	20
13	do	do	Treated	do	do	do	do	do	do	1	do	20
14	Mixed seed, 1 per cent rusted kernels	May 18	Untreated	5 rows 25 feet long	May 21	July 15	June 12	July 16	June 20	5	do	5
15	do	do	Treated	do	do	do	do	do	do	7	do	5
16	Same as No. 3	do	Untreated	do	do	do	do	July 15	do	2	do	1—
17	Same as No. 1	do	do	do	do	do	do	July 16	do	3	do	5—
18	Same as No. 6	do	do	do	May 22			July 10[a]	do	5	do	8—

[a] Two sori appeared on one plant in this plat on this date.

Nine different varieties of wheat seed were sown, and the plants were examined for rust every four or five days. Leafrust appeared on all the plots on June 5, and stemrust appeared from July 17 to July 29. Johnson sums up the results as follows: Rusted durum, Fife, and Bluestem kernels produced plants showing no earlier or more severe development of rust than adjacent plants from clean, uninfected seed.

EXPERIMENT 2.—On April 12, 1916, rusted kernels of wheat were sown in separate flats in the greenhouse. About 25 kernels were used from each of the following varieties: Allora (C. I. No. 1698), Kubanka (C. I. No. 1440), and Marquis (C. I. No. 3641). These flats were transferred to the pathological garden May 11, and were at that time in the fifth or sixth leaf. They were headed about June 22, and stemrust did not develop upon them until July 13, when a few leaves of the Marquis wheat, which still remained green, bore sori of *Puccinia graminis*. It will be noted by reference to Table IV that this was about the date upon which stemrust developed upon wheat in the field plots and was indeed about the date when stemrust appeared upon all the wheat in the vicinity. The season was very backward, and rust did not make its appearance nearly so early as usual.

HISTOLOGY OF SEEDS AND SEEDLINGS

HISTOLOGY OF SEED.—The general appearance of the exterior of wheat kernels infected with stemrust has been previously described. In order to examine the interior of these kernels two methods were found to be fairly satisfactory: One, in which the grains of wheat were boiled in water and then sectioned on the freezing microtome; the other, a modification of the glycerin method described by Howard (*13*). This latter method was found to be satisfactory, and good sections of mature wheat kernels were obtained. After sectioning, Pianeze stain was used with good results.

When sections of infected kernels were examined with a microscope it was found that not all the sori appeared upon the surface. In some instances the entire hilar region of the kernel was found to be filled with sori, of which from 1 to 12 were found in a single kernel. These sori often were found facing inward against the aleurone layer which was very much distorted by the pressure (Pl. 42). Other sori were found, nearly spherical in form, entirely embedded in the pericarp tissue. There seemed to be no regular arrangement, although the sori were often arranged in a circle around the hilum. This is what would be expected, for many of them undoubtedly were connected with infection on the rachilla before the kernel was broken away from the point of attachment. Plate 43 is a longitudinal section through the hilum of an infected kernel and shows the hilum nearly cut off by a large sorus, which probably was formed from several sori that had become confluent. Plate 44 is a cross

section of a mature wheat kernel with telia upon the ventral surface. Plate 45 is an enlarged portion of the same.

Internal rust sori of wheat kernels were noted and described also by Pritchard (*22*). More recently Colley (*8*) has listed 11 reports of internal rust sori upon various hosts. He concludes that these are rather common teratological phenomena having no especial morphological significance and can be expected to occur whenever the point at which the sorus begins to form is located beneath a layer of tissue which is too resistant for the sorus to break through. Plates 46 and 47 also show internal sori.

HISTOLOGY OF SEEDLINGS.—Rusted kernels of wheat were germinated under various conditions and for various lengths of time. These were fixed, sectioned, and examined for spread of infection from mycelium or spores embedded in the tissues. Various materials were used for fixing these young seedlings, but it was found that Juel's fixative penetrated the embryonic parts better than any other which was tried, although Flemming's medium fixative gave fairly satisfactory results. After sectioning, either triple stain with excess of Orange G or Pianeze stain was found to be satisfactory for differentiating host and fungus tissue.

Infected seed was germinated under the following conditions. Seed from lot 1 was germinated in compartments of an Altmann incubator kept at 2°, 12°, and 17° C., respectively. Part of these were fixed when the plumule was about ½ inch long, and the rest when the first leaf was just beginning to unfold. Seed from lot 2 was germinated in compartments of the Altmann incubator at temperatures of 2° alternated with 17° and 11° alternated with 21°. The experiments with lots 1 and 2 were conducted twice—once in November, 1915, and again in April, 1916, after the infected seed had been kept in a cool place over winter. Lot 3 was sown in pots which were placed in small chambers in the greenhouse where the soil temperature was kept between 11° and 15° by the use of ice. When the plants were about 3 or 4 inches tall they were fixed, and a portion of each was sectioned and examined. Lot 4 was germinated and buried out of doors in the ground at seeding time in the spring. The plants were treated as were those in lot 3.

Hundreds of sections were prepared from the material described above. In no case was there any positive evidence of spread of infection from the infected seed to the young plant.

Plates 46 and 47 illustrate this fact. Plate 46 represents a longitudinal section through a wheat embryo in a very early stage of development. There is no indication of any spread of rust mycelium from the sori seen in the infected hilar region at *x*. Plate 47 also represents a longitudinal section of a wheat embryo. In this case development has progressed considerably further than that shown on Plate 46. There is, however, absolutely not the slightest indication of spread of rust mycelium from the large sorus shown at *x*.

From all appearances the rust mycelium was dead in the sori of the germinated kernels shown in Plates 46 and 47. The same was true of the rust mycelium in wheat kernels that had been stored for some time. All such mycelium was devoid of normal protoplasmic content. This fact together with the apparent inability of this mycelium to spread to the developing seedling indicates clearly to the writer that this mycelium was dead. In fact, only in fresh kernels which were not fully matured were any living rust mycelia found. Numerous efforts were made also to germinate the teliospores found in sori upon the hilar portions of wheat kernels, but all were unsuccessful.

Hyphae of other organisms were present in abundance everywhere in the pericarp of many kernels and in some cases were found to penetrate the embryo. These hyphae were much larger and of an appearance different from the rust hyphae found at the base of the sori in the hilar portions of the kernels, as previously described. They penetrated directly through the cell walls of the host and broke down the cell structure to a much greater extent than rust infection was found to do. Plate 48 shows an oblique longitudinal section of a secondary root of a wheat seedling being invaded by this type of parasite. This was probably some species of Helminthosporium, for typical Helminthosporium spores were found on the germ end of the kernel from which this section was made. Mycelium of the same type was found in the root, stem, and sheath of a number of seedlings which were grown from kernels of wheat having a distinct browning of the hilar ends somewhat similar to the general appearance of rust-infected kernels. It seems not entirely unlikely, therefore, that the apparently similar mycelium referred to by Pritchard (*21*) may have been of this type, especially since he states that the mycelium he noted also was intracellular.

The writer did not find any "palmella-like" developments from the teliospores, as described by Pritchard. However, no seed over 1 year old was used, and since Pritchard used seed 5 years old this may to some extent account for the difference.

SUMMARY

(1) Uredinia and telia of *Puccinia graminis tritici* Erikss. and Henn. have been found embedded in the pericarp on the hilar end of kernels of wheat and sometimes along the ventral groove as far up as the middle of the kernel. Infected kernels have black hilar ends, and groups of telia appear as shining black specks under either the hand lens or the binocular microscope.

(2) Only a small percentage of infection was found by examination of the hundreds of samples of wheat from the crops of 1915 and 1916. A little over 1 per cent was the largest quantity found in any sample. The durum wheats were found most commonly infected.

(3) Infection undoubtedly spreads to the kernel from original infection on the rachis, rachilla, or glumes.

(4) The germinating power of the seed apparently is not impaired by this rust infection.

(5) When rusted kernels of wheat were sown in the field, no earlier or more severe rust infection occurred on the resulting plants than on those grown in adjacent plots which were sown either with clean seed or with rust-infected seed which had been treated with the modified hot-water treatment.

(6) More than 2,500 plants were grown from rusted seed in a specially constructed room in the pathological greenhouse at the University of Wisconsin, and no rust infection appeared upon any of them at any time. The conditions of growth of these plants were normal, and they produced plump grain.

(7) No spread of infection from the pericarp to the young plant was found by examination histologically, although infected seed were germinated under various conditions, simulating as nearly as possible natural conditions in the field.

(8) No infection appeared upon plants grown from seed which had been covered with viable urediniospores of stemrust before sowing.

(9) The results of the experimental work here reported indicate that stemrust is not transmitted from one wheat crop to the next by means of infected seed grain. Further, in the writer's judgment, the occurrence of stemrust sori in the pericarp of the caryopses of grains and grasses has no especial significance, but the infection spreads to these tissues just as it does from an infection point in any of the vegetative parts of the plant.

LITERATURE CITED

(1) BEAUVÉRIE, Jean.
1913. FRÉQUENCE DES GERMES DE ROUILLE DANS L'INTÉRIEUR DES SEMENCES DE GRAMINÉES. Compt. Rend. Acad. Sci. [Paris], t. 157, no. 18, p. 787–790.

(2) ——
1914. LES GERMES DE ROUILLES DANS L'INTÉRIEUR DES SEMENCES DE GRAMINÉES. *In* Rev. Gén. Bot., v. 25 (bis), p. 11–27, illus.

(3) BLARINGHEM, T.
1914. SUR LA PROPAGATION DES ROUILLES DE CÉRÉALES EN SUEDE ET EN FRANCE. Bul. Soc. Bot. France, t. 61, no. 1/3, p. 86–94.

(4) BOLLEY, H. L.
1898. EINIGE BEMERKUNGEN ÜBER DIE SYMBIOTISCHE MYKOPLASMATHEORIE BEI DEN GETREIDEROST. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, p. 887–896, 1 fig.

(5) —— and PRITCHARD, F. J.
1906. RUST PROBLEMS; FACTS, OBSERVATIONS AND THEORIES. POSSIBLE MEANS OF CONTROL. N. Dak. Agr. Exp. Sta. Bul. 68, p. 607–672, 30 fig.

(6) CARLETON, M. A.
1904. INVESTIGATIONS OF RUSTS. U. S. Dept. Agr. Bur. Plant Indus. Bul. 63, 32 p., 2 col. pl.

(7) COBB, N. A.
1902. COMPARATIVE OBSERVATIONS ON THE BRUSH OF ABOUT FIFTY VARIETIES OF WHEAT. *In* Agr. Gaz. N. S. Wales, v. 13, no. 6, p. 647–649, 3 fig.

(8) COLLEY, R. H.
1917. DISCOVERY OF INTERNAL TELIA PRODUCED BY SPECIES OF CRONARTIUM. *In* Jour. Agr. Research, v. 8, no. 9, p. 329–332, pl. 88. Literature cited, p. 332.

(9) ERIKSSON, Jakob.
1897. DER HEUTIGE STAND DER GETREIDEROSTFRAGE. *In* Ber. Deut. Bot. Gesell., Bd. 15, Heft 3, p. 183–194. Reprinted.

(10) ——— and HENNING, Ernst.
1896. DIE GETREIDEROSTE ... 463 p., 5 fig., 13 col. pl. Stockholm. Literaturverzeichnis, p. 446–457.

(11) GROVE, W. B.
1913. THE BRITISH RUST FUNGI ... 412 p., illus. Cambridge, [Eng.]. Bibliography, p. 393–397.

(12) HAYMAN, J. M.
1907. RUST IN WHEAT. *In* Rpt. Cawnpore Agr. Sta. United Prov. [India], 1907, p. 54–57.

(13) HOWARD, B. J.
1903. SECTIONING OF WHEAT KERNELS. *In* Jour. Appl. Micros. and Lab. Methods, v. 6, no. 9, p. 2498–2499, 1 fig.

(14) JACZEWSKI, A. von.
1910. STUDIEN ÜBER DAS VERHALTEN DES SCHWARZROSTES DES GETREIDES IN RUSSLAND. *In* Ztschr. Pflanzenkrank., Bd. 20, Heft 6, p. 321–359, 8 fig.

(15) JOHNSON, Edw. C.
1911. FLORET STERILITY OF WHEATS IN THE SOUTHWEST. *In* Phytopathology, v. 1, no. 1, p. 18–27.

(16) KLEBAHN, H.
1899. EIN BEITRAG ZUR GETREIDEROSTFRAGE. *In* Ztschr. Pflanzenkrank., Bd. 8, Heft 6, p. 321–342, illus., pl. 6.

(17) LAGERHEIM, G.
1893. UEBER DAS VORKOMMEN VON EUROPÄISCHEN UREDINEEN AUF DER HOCHEBENE VON QUITO. *In* Bot. Centbl., Bd. 54, No. 11, p. 324–327.

(18) MCALPINE, D.
1906. RUSTS OF AUSTRALIA ... 349 p., 5 pl. (partly col.) Melbourne. Literature, p. 213–221.

(19) MADDOX, Frank.
1897. NOTES AND RESULTS ON AGRICULTURAL EXPERIMENTS CARRIED OUT UNDER THE AUSPICES OF THE COUNCIL OF AGRICULTURE OF TASMANIA, AT EASTFIELD, NEWNHAM. 90 p., illus. Launceston.

(20) MASSEE, Geo.
1899. THE CEREAL RUST PROBLEM.—DOES ERIKSSON'S MYCOPLASMA EXIST IN NATURE? *In* Nat. Sci., v. 15, no. 93, p. 337–346. References, p. 346.

(21) PRITCHARD, Frederick J.
1911. A PRELIMINARY REPORT ON THE YEARLY ORIGIN AND DISSEMINATION OF PUCCINIA GRAMINIS. *In* Bot. Gaz., v. 52, no. 3, p. 169–192, pl. 4. Literature cited, p. 190–192.

(22) ———
1911. THE WINTERING OF PUCCINIA GRAMINIS TRITICI E. & H. AND THE INFECTION OF WHEAT THROUGH THE SEED. *In* Phytopathology, v. 1, no. 5, p. 150–154, 2 fig., pl. 22.

(23) SMITH, W. G.
1885. CORN MILDEW. *In* Gard. Chron., n. s. v. 24, no. 608, p. 245, fig. 53.

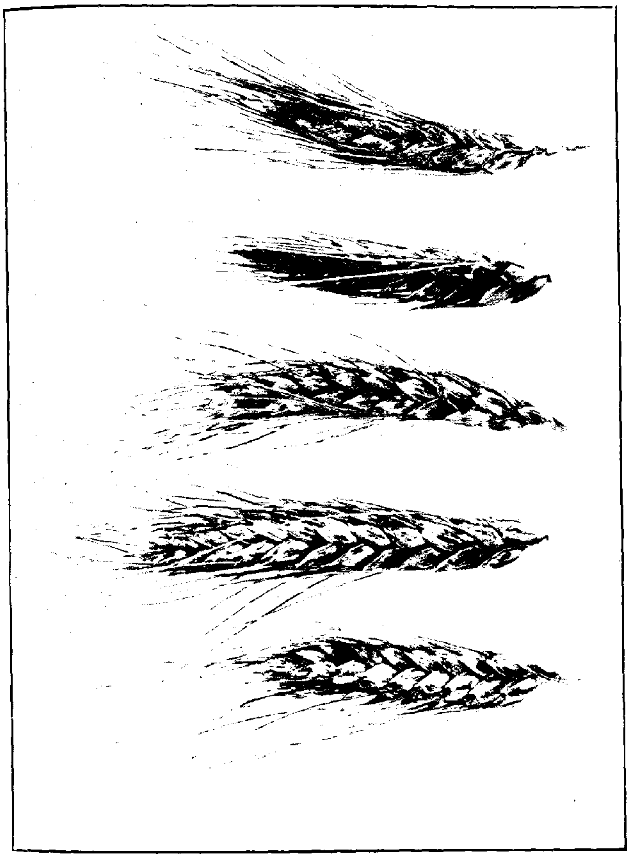
(24) ———
1886. CORN MILDEW AND BARBERRY BLIGHT. *In* Gard. Chron., n. s. v. 25, no. 636, p. 309–310, fig. 58–60.

(25) WARD, H. Marshall.
1903. "ON THE HISTOLOGY OF UREDO DISPERSA, ERIKSS., AND THE 'MYCOPLASM' HYPOTHESIS." (Abstract.) *In* Proc. Roy. Soc. London, v. 71, no. 473, p. 353–354.

(26) ZUKAL, Hugo.
1899. UNTERSUCHUNGEN ÜBER DIE ROSTPILZKRANKHEITEN DES GETREIDES IN OESTERREICH-UNGARN. (I. REIHE). *In* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Abt. 1, Bd. 108, Heft 6/7, p. 543–562.

PLATE 38

Heads of Kubanka durum wheat heavily infected with stemrust. Collected at Dickinson, N. Dak., in 1916.



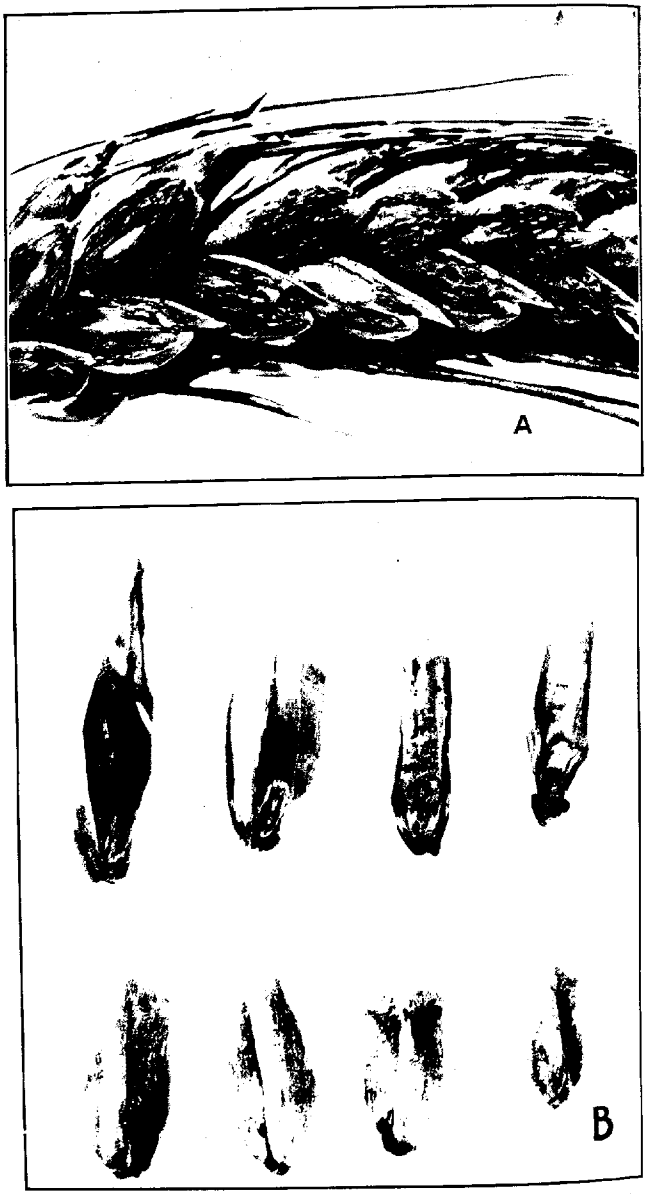

A
B

PLATE 39

A.—Portion of one of the heads shown in Plate 38. ×3.6.

B.—Wheat kernels showing typical stemrust infection. Abundant infection occurs at the base of the attached paleae on the upper row of kernels. In the lower row rust sori occur at the hilar end and along the ventral groove. ×6.

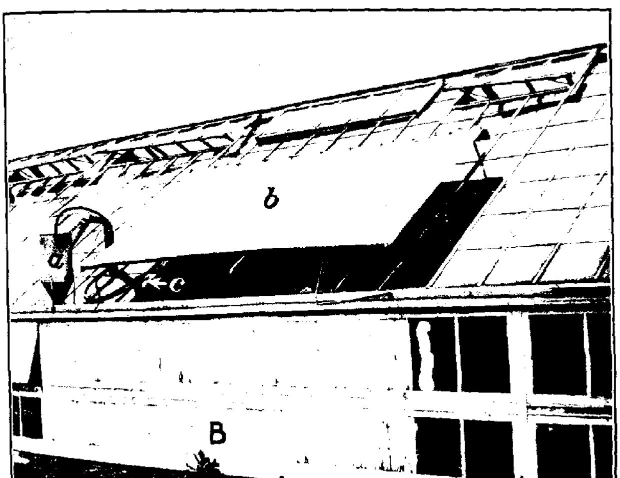
PLATE 40

Exterior view of isolated room in the pathological greenhouse at the University of Wisconsin, showing (*a*) the exterior portion of air-washing apparatus used to wash all air drawn into the room, (*b*) the canvas curtain used for shading on warm days, and (*c*) the sprinkling attachment used to throw spray of water over the roof to aid in keeping the room cool. (See fig. 1 and description of apparatus in text.)

A.—Greenhouse with canvas curtain rolled up.

B.—Greenhouse with canvas curtain rolled down.


b
a
c
A


b
a
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B

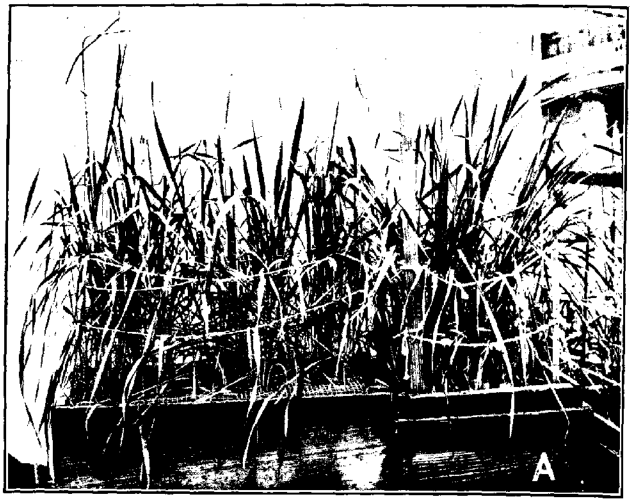

PLATE 41

A.—Photograph of wheat grown in flats in isolated room in greenhouse at the University of Wisconsin. The healthy, vigorous growth of the plants indicates that normal growing conditions prevailed in the greenhouse.

B.—Same plants as A, when well headed. Plump kernels of wheat were harvested from all these plants.

PLATE 42

Longitudinal section through hilar portion of an immature wheat kernel, showing sorus of stemrust. Abundance of living rust mycelium is shown at the base of the sorus. Note (at left) the aleurone layer which has been forced inward. No evidence of mycelial penetration into aleurone layer of cells. $\times 245$.

PLATE 43

Longitudinal section through the hilum of a wheat kernel infected with stemrust, showing unusually large internal sori extending nearly across the kernel. Both external and internal sori are shown. No evidence of invasion of aleurone cells was found. ×85.

PLATE 44

Cross section of a mature wheat kernel infected with stemrust, showing telia in the ventral groove. Note the normal appearance of the cells of the aleurone layer immediately beneath the sori. ×50.

PLATE 45

Enlarged portion of section shown in Plate 44, showing telia on surface of ventral groove. No evidence of penetration into aleurone cells exists. $\times 278$.

PLATE 46

Longitudinal section of embryo of germinated wheat kernel showing large internal rust at *x* in hilar tissue at base of embryonic tissue. Hundreds of such sections were examined without evidence of spread of rust infection to the embryo. ×65.

PLATE 47

Longitudinal section of the embryo further advanced in development than that shown in Plate 50. Internal hilar sorus shown at *x*. No evidence of infection of embryonic tissues. ×67.

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PLATE 48

Longitudinal section through young secondary root of wheat embryo, showing presence of intracellular mycelium. The fungus here is probably a species of Helminthosporium. This mycelium is larger and more vacuolated and breaks down the cells of the host much more completely than does the rust mycelium. (See Pl. 42 for comparison.) ×255.

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